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Intracellular protein topogenesis

(protein translocation across membranes/protein integration into membranes/posttranslocational sorting/topogenic sequences/phylogeny of membranes and compartments)

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ABSTRACT **Concurrently with or shortly after their synthesis on ribosomes, numerous specific proteins are unidirectionally translocated *across* or asymmetrically integrated *into* distinct cellular membranes. Thereafter, subpopulations of these proteins need to be sorted from each other and routed for export or targeted to other intracellular membranes or compartments. It is hypothesized here that the information for these processes, termed "protein topogenesis," is encoded in discrete "topogenic" sequences that constitute a permanent or transient part of the polypeptide chain. The repertoire of distinct topogenic sequences is predicted to be relatively small because many different proteins would be topologically equivalent—i.e., targeted to the same intracellular address. The information content of topogenic sequences would be decoded and processed by distinct effectors. Four types of topogenic sequences could be distinguished: signal sequences, stop-transfer sequences, sorting sequences, and insertion sequences. *Signal* sequences initiate translocation of proteins across specific membranes. They would be decoded and processed by protein translocators that, by virtue of their signal sequence-specific domain and their unique location in distinct cellular membranes, effect unidirectional translocation of proteins across specific cellular membranes. *Stop-transfer* sequences interrupt the translocation process that was previously initiated by a signal sequence and, by excluding a distinct segment of the polypeptide chain from translocation, yield asymmetric integration of proteins into translocation-competent membranes. *Sorting* sequences would act as determinants for posttranslocational traffic of subpopulations of proteins, originating in translocation-competent donor membranes (and compartments) and going to translocation-incompetent receiver membranes (and compartments). Finally, *insertion* sequences initiate unilateral integration of proteins into the lipid bilayer without the mediation of a distinct protein effector. Examples are given for topogenic sequences, either alone or in combination, to provide the information for the location of proteins in any of the intracellular compartments or for the asymmetric orientation of proteins and their location in any of the cellular membranes. Proposals are made concerning the evolution of topogenic sequences and the relationship of protein topogenesis to the precellular evolution of membranes and compartments.**

"Intracellular protein topogenesis" is used here as a categorical term for those intracellular processes that occur concomitantly with or shortly after synthesis of proteins on ribosomes and that result in the unidirectional translocation of the proteins *across* or asymmetric integration of them *into* translocation-competent membranes as well as their subsequent posttranslocational pathway to other (translocation-incompetent) cellular membranes and compartments. Recent *in vitro* studies on the early events (translocation and integration) in the topogenesis of a number of specific proteins of distinct cellular topology (reviewed in ref. 1) have provided important information. An attempt is made in this paper to integrate this information on the early events in topogenesis into a conceptual framework, to extrapolate from there to later events (posttranslocational pathways), and thus to formulate a general hypothesis on intracellular protein topogenesis. This hypothesis predicts that the determinants for intracellular protein topogenesis reside in *discrete* segments of each polypeptide chain. These segments, termed "topogenic sequences," are permanent or transient features of the protein and are characterized by their redundancy, being shared by the many structurally otherwise different proteins whose common denominator is an identical topogenesis. Furthermore, the phylogeny of biological membranes, of cells, and of cellular compartments will be considered with respect to protein topogenesis.

Translocation of proteins across membranes

Translocation is understood here as transport of an entire polypeptide chain across one (or two) membrane(s), proceeding unidirectionally from the protein biosynthetic compartment. Not considered in this category are "ectopically" synthesized proteins (e.g., toxins such as the colicins or diphtheria toxin) although their entry into cells may also proceed via translocation across the plasma membrane.

Two essential tenets have to be considered in any hypothesis on protein translocation. First, the permeability barrier of the membrane is reversibly and selectivity modified for the passage of each translocated polypeptide chain while being maintained for other solutes. Second, the species of protein to be translocated as well as the type of translocation-competent membrane(s) engaged in its translocation is highly specific. The detailed proposals (1) that have been made so far to satisfy these tenets can be stated in an abbreviated version: all polypeptide chains to be translocated contain the information for their translocation in a discrete portion of the newly synthesized chain, termed the "signal" sequence; the signal sequence is addressed to specific integral membrane proteins that effect translocation of the polypeptide chain.

Two modes of translocation have been distinguished. In one mode (Fig. 1 *Top*), first described for secretory proteins (2, 3), translocation is cotranslational—i.e., strictly coupled to translation. In the other mode of translocation, first described for a cytosol-synthesized chloroplast stroma protein (4–6) and then for other cytosol-synthesized proteins that are imported into peroxisomes (7) or the mitochondrial matrix (8), translocation is posttranslational—i.e., it is uncoupled from translation. Posttranslational translocation can proceed across either one membrane or two membranes (Fig. 1 *Middle* and *Bottom*).

Common to the proposed models for the two modes of translocation (Fig. 1) is the idea that a bivalent ligand would be able to recruit and to "crosslink" corresponding receptor domains of translocator subunits into a functional translocator. In cotranslational translocation (Fig. 1 *Top*) this bivalent ligand would be represented by the signal sequence and a site on the

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Abbreviations: See Table 1 for codes for membranes; IMPs, integral membrane proteins.

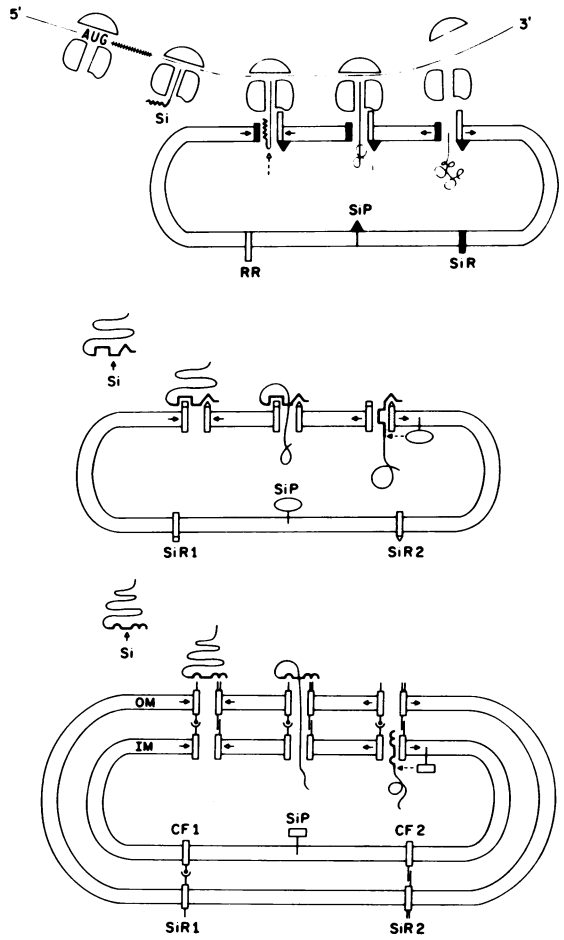

FIG. 1. Schematic models for cotranslational translocation (*Top*) and posttranslational translocation across *one* membrane (*Middle*) or *two* membranes (*Bottom*). Translocator subunits are represented as bilateral (see Fig. 2) integral membrane proteins with their receptor domains asymmetrically exposed (or accessible) only at that side of the membrane that faces the protein biosynthetic compartment (prokaryotic and eukaryotic cytoplasm, mitochondrial matrix, chloroplast stroma). According to their receptor domains the translocator subunits are referred to as signal receptor (SiR) or ribosome receptor (RR). Signal receptors may be linked to corresponding coupling factors (CF) to achieve simultaneous posttranslational translocation across the outer (OM) and inner (IM) membrane (*Bottom*). As an alternative to this "gap junction" model (*Bottom*), posttranslational translocation could proceed consecutively, first across the outer membrane and then across the inner membrane by using two signal sequences, one addressed to the outer membrane (identical to that of proteins translocated only across the outer membrane) and another one addressed to the inner membrane. Arrows in the plane of the membrane indicate assembly or disassembly of the translocator. Signal peptidase (SiP) is arbitrarily indicated either to be closely associated with (*Middle* and *Bottom*) or to be an integral part of (*Top*) the assembled translocator. The signal peptidase cleavage site between the signal sequence (Si) and the remainder of the polypeptide chain is indicated by a dashed arrow.

large ribosomal subunit. In posttranslational translocation, on the other hand, the ribosome would not contribute and the bivalent ligand would be represented instead by *two* distinct domains in the signal sequence (Fig. 1, *Middle* and *Bottom*). The translocator would be disassembled by disassociation and lateral diffusion of the subunits in the plane of the membrane.

Cellular membranes that are competent (1) for cotranslational or posttranslational translocation are listed in Table 1. It is envisioned that each of the nine listed membranes (or membrane pairs) is endowed with only *one* distinct translocator (present in multiple copies) that specifically decodes only *one* type of signal sequence. Accordingly, a tentative number of translocator-specific signal sequences (or signal sequence-specific translocators) would be: one in prokaryotic cells, five in animal cells, and eight in plant cells (having both mitochondria and chloroplasts).

Table 1. Cellular membranes proposed to be endowed with a transport system (translocator) for unidirectional translocation of nascent or newly synthesized proteins

Translocation mode	Membrane	Code
Cotranslational	a. Prokaryotic plasma membrane	PPM
	b. Inner mitochondrial membrane	IMM
	c. Thylakoid membrane	TKM
	d. Rough endoplasmic reticulum	RER
Posttranslational (across *one* membrane)	e. Outer mitochondrial membrane	OMM
	f. Outer chloroplast membrane	OCM
	g. Peroxisomal membrane	PXM
Posttranslational (across *two* membranes)	h. Mitochondrial envelope	MEN
	i. Chloroplast envelope	CEN

Each of the translocation-competent membranes (1) listed here (a–i) is proposed to contain only *one* distinct "translocator" (in multiple copies). Each translocator responds to *one* type of signal sequence. Translocation can proceed across a *single* membrane (a–g) or across *two* membranes (h and i), cotranslationally (a–d) or posttranslationally (e–i). Suggested abbreviations for these translocation-competent membranes might serve as useful codes. For example, a signal sequence (Si) addressed to the rough endoplasmic reticulum (RER), to the chloroplast envelope (CEN), etc., might be designated Si(RER), Si(CEN), etc. Likewise, a particular signal receptor (SiR) or signal peptidase (SiP) could be classified as SiR(RER), SiR(CEN), or SiP(RER), SiP(CEN), etc. Ribosome receptors (RR) are limited to membranes with cotranslational translocators (a–d). Again, they could be classified as RR(PPM), RR(IMM), etc.

A signal sequence may or may not be cleaved after translocation. Its domain for cleavage by signal peptidase may be altered independently from its domain for translocation [uncleaved signal sequence (9, 10)]. The existence of an internal signal sequence addressed to the RER has recently been demonstrated for chicken ovalbumin (10). Primary structure information is now available for the signal sequence addressed to RER (many examples are summarized in ref. 1), for the signal sequence addressed to PPM (a few examples are summarized in ref. 1), and for one example of a signal sequence addressed to CEN (11).

Integration into membranes

Many integral membrane proteins (IMPs) require selective translocation of one or more hydrophilic segment(s) of the polypeptide chain in order to acquire their characteristic asymmetric orientation. How could a selective translocation of discrete segment(s) of the polypeptide chain be accomplished?

In considering hypothetical solutions to this problem it is useful to seek an arbitrary definition of the theoretically possible modes of orientation of the polypeptide chain of IMPs with respect to the hydrophobic core and the hydrophilic environment of the lipid bilayer. IMPs can be classified as monotopic, bitopic, and polytopic (Fig. 2). The polypeptide chain of monotopic IMPs exhibits unilateral topology—i.e., each molecule possesses hydrophilic domain(s) exposed to the hydrophilic environment on only *one* side of the membrane. The polypeptide chain of bitopic and polytopic IMPs is bilateral in

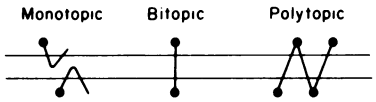

FIG. 2. Classification of integral membrane proteins (IMPs) as monotopic, bitopic, and polytopic. The hydrophobic boundary of the lipid bilayer is indicated by two parallel lines. Solid circles on polypeptide chains indicate major hydrophilic domains. The hydrophilic domain of an individual *monotopic* IMP is exposed only on one side of the lipid bilayer. A hydrophobic domain is indicated to anchor the polypeptide chain to the hydrophobic core of the lipid bilayer. A monotopic IMP may contain several hydrophilic and hydrophobic segments alternating with each other (not indicated here). However, all hydrophilic domains are unilaterally exposed. The polypeptide chain of *bitopic* IMPs spans the lipid bilayer once and contains a hydrophilic domain on each side of the membrane. In variants of bitopic IMPs (not indicated), the bilateral hydrophilic domains could be further subsegmented by interspersed hydrophobic domains that are capable of monotopic integration. The polypeptide chain of *polytopic* IMPs spans the membrane more than once and contains multiple hydrophilic domains on both sides of the membrane. The existence of polytopic IMPs remains to be demonstrated. Two structurally monotopic IMPs located on opposite sides of the membrane could interact via their hydrophobic anchorage domains and form a functionally bilateral ensemble.

nature, containing two or multiple hydrophilic domains, respectively, exposed on opposite sides of the membrane.

It is proposed that all of these orientations could be accomplished by invoking only two additional types of topogenic sequences, termed "*stop-transfer* sequences" and "*insertion* sequences."

The *stop-transfer* sequence was proposed to contain the information to interrupt the chain translocation that was initiated by a signal sequence—e.g., by effecting premature disassembly of the translocator into subunits (12–14). Because the order of translocation of the polypeptide chain could be expected to proceed asymmetrically in both cotranslational *and* posttranslational translocation, stop-transfer sequences would be effective means for asymmetric integration of IMPs by both modes of translocation. There could be as many translocator-specific stop-transfer sequences as there are translocator-specific signal sequences. On the other hand, there could be only one stop-transfer sequence addressed to one component common to all translocators.

An *insertion* sequence contains the information to effect *monotopic* integration of the polypeptide chain into the lipid bilayer *without* the mediation of a protein translocator. The choice of membrane for insertion may depend on the affinity of a monotopic IMP for another membrane protein.

Examples for programs of various topogenic sequences that could result in monotopic, bitopic, and polytopic orientation of the polypeptide chain are given in Fig. 3. It is clear from these examples that the integration of most proteins into the membrane requires a signal sequence and a translocator, except for one subgroup of monotopic IMPs (see Fig. 3, upper left example). Thus, most IMPs can be integrated directly only into translocation-competent membranes. Because the translocators themselves are likely to consist of IMPs (see Fig. 1) that require translocation for their integration into the membrane, it follows that Virchow's paradigm on the ontogeny of cells could be extended to membranes and paraphrased to *omnis membrana e membrana.*

Phylogeny of membranes, protein translocation, and compartments

How then could biological membranes with their characteristic asymmetry of proteins and lipids have evolved if their assembly was dependent on the developement of a mechanism for protein translocation across the lipid bilayer? In an attempt to retrace the "phylogeny" of membranes one could distinguish between precellular and cellular stages of evolution. Starting with lipid vesicles (Fig. 4), the first step in the precellular evolution of biological membranes may have been monotopic integration of proteins into the outer leaflet of lipid vesicles via insertion sequences. Such vesicles could have functioned as capturing devices to collect, on their outer surface, components involved in replication, transcription, and translation as well as metabolic enzymes present in the surrounding medium (Fig. 4*A*). In this way, much of the precellular evolution and assembly of macromolecular complexes (such as the ribosome) may have proceeded on the surface of these vesicles. By vesicle fusion, larger vesicles containing a synergistic assortment of functions could have evolved, resulting essentially in the formation of "inside-out cells" (Fig. 4 *A* and *B*).

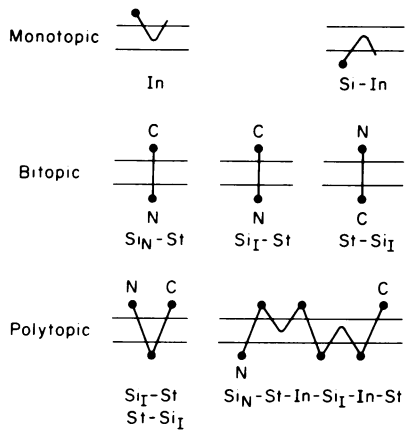

FIG. 3. Program of topogenic sequences for the asymmetric integration into membranes of some representative examples of monotopic, bitopic, and polytopic IMPs. Hydrophobic boundary of lipid bilayer is indicated by two parallel lines, with upper line facing the protein biosynthetic compartment. Solid circles represent major hydrophilic domains which, when indicated, contain amino (N) or carboxy (C) terminus of the polypeptide chain. Topogenic sequences are: insertion sequence (In), signal sequence (Si), and stop-transfer sequence (St). Si_N and Si_I indicate amino-terminal and internal signal sequences, respectively. Examples given here (except for monotopic IMP at upper left) are for cotranslational integration into RER. Similar programs are conceivable also for cotranslational integration into PPM, IMM, and TKM as well as for posttranslational integration into PXM, OMM, OCM, IMM [using Si(MEN)], and ICM/TKM [using Si(CEN)]. An attempt has been made to list topogenic sequences in order of their location along the polypeptide chain starting from the amino terminus. The problems encountered in predicting the order relate to uncertainties as to the order of chain translocation. In particular, in the case of an internal signal sequence (Si_I) there are several possibilities depending on the order of translocation (10). The orientation of a polytopic IMP such as indicated at the lower right is entirely hypothetical and is illustrated here only to indicate how such a polypeptide chain could be integrated into the membrane by a program of multiple topogenic sequences.

Concurrent with the development of such inside-out cells could have been the development of mechanisms for the translocation of proteins, thus providing the opportunity to segregate proteins, to colonize (with monotopic IMPs) the interior leaflet of the vesicle's lipid bilayer, and to integrate bitopic IMPs. Toward this end, the ribosome–membrane junction could have been remodeled and the insertion sequence could have evolved into a signal sequence so as to achieve first a cotranslational mode of translocation. The development of the stop-transfer sequence (perhaps as a variant of the signal sequence) to integrate bitopic IMPs may have concluded the precellular evolution of the cotranslational mechanism for the assembly of membranes. The posttranslational mode of trans-

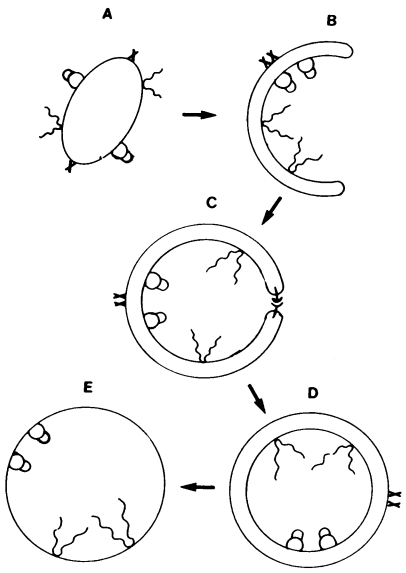

FIG. 4. Schematic illustration of various theoretical stages of precellular evolution on the surface of vesicles culminating in the formation of a primordial cell. (*A*) Vesicles containing monotopic IMPs (not indicated) able to bind various macromolecules (X) and macromolecular complexes, among them chromatin and ribosomes. (*B*) Nonrandom distribution of bound components on the vesicle surface and beginning invagination. (*C*) Formation of a "gastruloid" vesicle, perhaps able to open and to close via protein–protein interaction of bitopic IMPs at its orifice. (*D*) Fusion at the orifice, resulting in a primordial cell delimited by two membranes. (*E*) Loss of the outer membrane. *D* could have evolved into Gram-negative bacteria and *E* into Gram-positive bacteria and eukaryotic cells (see Fig. 5).

location may have evolved (1) from the cotranslational mode by transposing the information that is contained in the ribosome and adding it to the signal sequence for cotranslational translocation (see Fig. 1). The integration of bitopic IMPs into the lipid bilayer permitted the development of transport systems and signaling systems. This set the stage for evolution to continue *within* a closed system (the primordial cell) effectively sealed from some of the hazards of the surrounding medium by the lipid bilayer but able to communicate with the outside via the lipid bilayer-integrated transport and signaling systems. This primordial cell (Fig. 4*D*) may have possessed two membranes, a plasma membrane delimiting the newly generated endoplasmic compartment, and an outer membrane enclosing a periplasmic space that represents the remnant of the intravesicular space of the inside-out cell. Subsequent elimination of the outer membrane would have yielded a cell with only one membrane (Fig. 4*E*), the plasma membrane, and one compartment, the endoplasmic compartment. All other biological membranes could have originated either directly or indirectly from this primordial plasma membrane.

The membranes of eukaryotic cells could be traced to two distinct sources (Fig. 5). One would be the cell's *own* primordial plasma membrane, generating by invagination various "orthoplasmic" membranes which delimit a new intracellular compartment, the ectoplasmic compartment (Fig. 5*A*). The other source (based on the theory of endosymbiosis; see ref. 15) would be the plasma membrane of a *foreign* symbiotic cell (at a "prenuclear" stage of evolution) which, after being interiorized, would give rise to "xenoplasmic" membranes delimiting a xenoplasmic subcompartment within the ectoplasmic compartment (Fig. 5*B*).

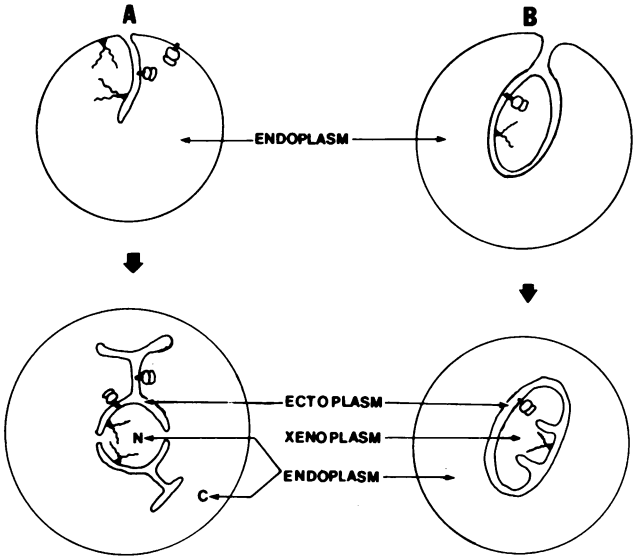

FIG. 5. Schematic illustration of the evolution of intracellular membranes and compartments. (*A*) Aggregation of certain membrane functions in the plane of the pluripotent plasma membrane. Nonrandom removal of these functions from the plasma membrane by invagination and fission results in the formation of a nuclear envelope (pore complexes omitted) continuous with the endoplasmic reticulum (rough and smooth) and generates an ectoplasmic compartment. The endoplasmic compartment is thereby subdivided into nucleoplasm (N) and cytoplasm (C). Note, however, that N and C remain connected via nuclear pores that do not have a membraneous barrier. Other intracellular membranes that are distinct from the endoplasmic reticulum, such as lysosomal, peroxisomal, and Golgi complex membranes, also could have developed by invagination from the plasma membrane or could be outgrowths of the endoplasmic reticulum. (*B*) Symbiotic capture of another cell, generating an additional xenoplasmic compartment. Green plant cells have two such xenoplasmic compartments (mitochondrial matrix and chloroplast stroma). Only the inner mitochondrial membrane and the inner chloroplast membrane (including derived thylakoid membrane) would be of xenoplasmic origin, whereas the outer mitochondrial and chloroplast membranes would be of orthoplasmic origin, like all other cellular membranes. The proposed terminology may be useful for describing the precise topology of IMPs (see Fig. 2). For example, monotopic IMPs of the thylakoid membrane may be exposed ectoplasmically (i.e., toward the intradisc space) or xenoplasmically (i.e., toward the stroma); bitopic IMPs of the outer mitochondrial membrane have an ectoplasmic and an endoplasmic domain, etc.

Posttranslocational pathways

The nonrandom removal of distinct membrane functions from a pluripotent primordial plasma membrane during evolution would generate a number of highly differentiated intracellular membranes that lack a translocator and that are physically not continuous (at least not permanently) with translocation-competent membranes. These translocation-incompetent membranes (or the subcompartments they enclose) therefore must receive their translocation-dependent, constitutive IMPs (or segregated proteins) from translocation-competent membranes (or subcompartments).

The most significant donor membrane (subcompartment) is the RER which probably supplies translocation-dependent proteins to essentially all orthoplasmic membranes and ectoplasmic subcompartments (16). Each of the receiving membranes presumably contains a set of IMPs that are *permanent residents* (either constitutive to a particular receiving membrane or shared by several other orthoplasmic membranes) and a set of *proteins in transit* [either on their way to their permanent residence or cycling between orthoplasmic membranes (e.g., carrier proteins, see below)].

Table 2. Alternate-choice programs of topogenic sequences for topologically equivalent proteins

Membrane	Bitopic IMPs	Content proteins
Peroxisomal	Si(PXM)-St	Si(PXM)
	Si(RER)-St-So	Si(RER)-So
Inner mitochondrial	Si(IMM)-St	Si(MEN)
	Si(MEN)-St	
Thylakoid	Si(TKM)-St	Si(TKM)
	Si(CEN)-St-So	Si(OCM)-So

Abbreviations as in Table 1; St, stop-transfer sequence; So, sorting sequence. Listed are programs only for bitopic IMPs and content proteins that are not integral membrane proteins. Alternate programs analogous to those shown for the *peroxisomal* membrane are theoretically possible also for the outer membrane of mitochondria and chloroplasts, whereby the "content" proteins would correspond to proteins that are located in the ectoplasmic compartment (intermembrane space) of mitochondria and chloroplasts (see Fig. 5). Likewise, a program analogous to that shown for the *inner mitochondrial* membrane is conceivable also for the inner membrane of chloroplasts. For the corresponding "content" proteins in the xenoplasmic compartment there most likely is no alternate program of topogenic sequences: proteins are synthesized either within the xenoplasmic compartment or imported via Si(MEN) or Si(CEN). The alternate programs for bitopic IMPs in the *thylakoid* membrane are similar to those in the inner chloroplast membrane, except that sorting sequences may be required for the program Si(CEN)-St to distinguish between those bitopic IMPs that remain in the inner membrane and those that continue (by invagination) to become residents of TKM. By the same token, one of the programs [Si(OCM)-So] for the corresponding "content" proteins in the intradisc space is based on the possibility that this space communicates transiently with the ectoplasmic space of chloroplasts.

The information for posttranslocational traffic could reside in one (or several) discrete segments of the polypeptide chain. Proteins with an identical travel objective could share this information. These sequences, termed "sorting sequences," would therefore constitute another group of topogenic sequences. Sorting sequences may be required not only for proteins that leave the RER but also for those that need to be anchored there.

It is possible, however, that individual proteins may be able to reach their target without a sorting sequence(s). They could do this merely by association with another protein (piggybacking) that is endowed with a sorting sequence(s). Likewise, sorting sequences (as defined here) may not be needed for the nonrandom distribution of proteins within physically continuous membranes. Protein–protein interactions to form large ensembles with a decreased rate of diffusion in the plane of the membrane and possibly anchored by cytoskeletal elements (17) could be responsible for the regional differences that are characteristic of continuous membranes.

Decoding of the information contained in the sorting sequences should be effected by specific proteins. For sorting sequences of bilateral *IMPs*, the effector may be represented by a few distinct peripheral membrane proteins. For sorting sequences of *soluble* proteins, such as lysosomal enzymes, the effector may be represented by a bilateral IMP that functions as a carrier protein shuttling back and forth between the donor and a receiver compartment. Its *ectoplasmic* domain may be able to bind reversibly to the sorting sequence(s) of lysosomal enzymes, and its *endoplasmic* domain may contain a sorting sequence for a cyclic traffic pattern between the donor (RER) and receiver compartments [the latter could be represented by a distinct portion of the endoplasmic reticulum from which primary lysosomes develop (18)]. A defect in the carrier could result in secretion of all lysosomal enzymes.

The need for sorting arose from the use of only one translocator for topologically different proteins. The reverse—namely, the potential to use more than one translocator for topologically equivalent proteins—may have arisen when certain membranes (see Table 1) acquired a posttranslational translocator. For example, there could be two programs of topogenic sequences for peroxisomal proteins (Table 2), both for the "content" proteins of the peroxisome and for those constitutive of the peroxisomal membrane (exemplified by bitopic IMPs). In reality, however, only one program for each group may exist, such as Si(PXM) for peroxisomal content proteins and Si(RER)-St-So for peroxisomal bitopic IMPs (7), with the alternate program either never developed or eliminated in evolution.

On the other hand, both programs indicated in Table 2 for the integration of bitopic IMPs into the inner mitochondrial membrane (or the inner membrane of chloroplasts) and into the thylakoid membrane are likely to exist.

Finally, if topogenic sequences behaved in evolution like "transposable" elements, one could conceive of "pleiotopic" proteins (1) that are similar in structure and function but different in topology. Pleiotopic proteins could have arisen by gene duplication and by the loss or acquisition (via transposition) of a topogenic sequence(s). Such processes may have been important (*i*) for achieving dichotomy in the posttranslocational pathway of proteins (e.g., secretory and lysosomal proteins) or (*ii*) for achieving either export or retention via binding to membranes (e.g., secreted or membrane-bound form of IgM heavy chains) or (*iii*) for diversifying the organellar distribution of proteins (e.g., some proteins that may occur both within peroxisomes and the mitochondrial matrix) or (*iv*) for anchoring polymeric structures in the membrane (e.g., free and membrane-bound forms of cytoskeletal proteins).

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